The Pan-Pacific Entomologist

VOL. 52 JULY 1976 No. 3

Multivariate Analysis of Geographic Variation in *Libellula luctuosa* Burmeister

(Odonata: Libellulidae)

ROSSER W. GARRISON

University of California, Berkeley

The study of intraspecific variation in dragonflies has rarely proceeded beyond the naming and describing of new taxa. This is partly due to the paucity of material at hand: dragonflies are seldom collected and the ranges even of many common species are poorly known. Most of the papers analyzing geographic variation in odonates (Bennefield, 1965; Huggins, 1927; Johnson, 1972) have involved tabulation of characters, with taxonomic decisions usually based on observable differences in features such as color, size, shape, or wing maculation. Multivariate methods of classification may be extremely helpful to the odonate systematist, especially when populations intergrade and variation among different characters is discordant. This paper analyzes geographic variation in a common and widespread species and compares the results of four multivariate methods of analysis for consistency.

Libellula luctuosa Burmeister is a large, dark dragonfly with black basal wing bands which occurs over most of the United States except the Great Basin and Florida. It occurs in British Columbia, Quebec, Manitoba, and Nova Scotia and is known in Mexico from Chihuahua and Durango. Variation in coloration, maculation, or size might be expected, since it ranges from mesic northeastern deciduous forests to cattle ponds of the hot, arid Southwest.

Three different phenotypes of *L. luctuosa* were subjectively recognized by the author; their distribution is shown in Figure 1. From southern Canada and the Atlantic coast to the eastern edge of the Great Plains, *L. luctuosa* is characterized by uniformly dark hind wing bands (Fig. 4a). From the edge of the Great Plains south through Oklahoma, eastern Colorado, Texas, New Mexico, southeastern Arizona, and Mexico, populations possess various degrees of hind wing clearing (Fig. 4a–d). Hagen (1861) described these paler individuals as a new species, *Libellula odiosa*, but subsequently (1875) reduced *L. odiosa* to a race

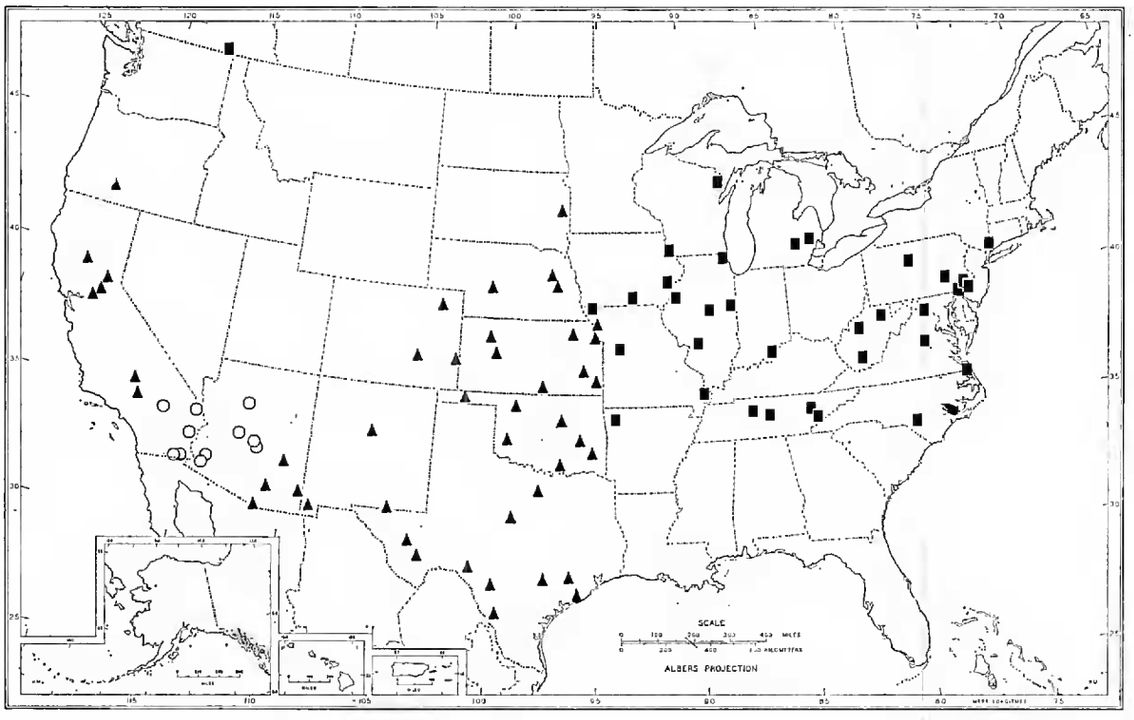

FIG. 1. Map of specimen localities, *Libellula luctuosa*. Solid squares = eastern luctuosa morphs, solid triangles = odiosa morphs, open circles = Colorado River phenotypes. More than one specimen may have been taken at some localities.

of *L. luctuosa*. Most authors since have considered *L. odiosa* a variety or synonym of *L. luctuosa* (Calvert, 1906; Needham and Westfall, 1955; Ris, 1910). These individuals are hereafter called odiosa, though the taxon is believed to be synonymous with eastern *L. luctuosa*. The odiosa

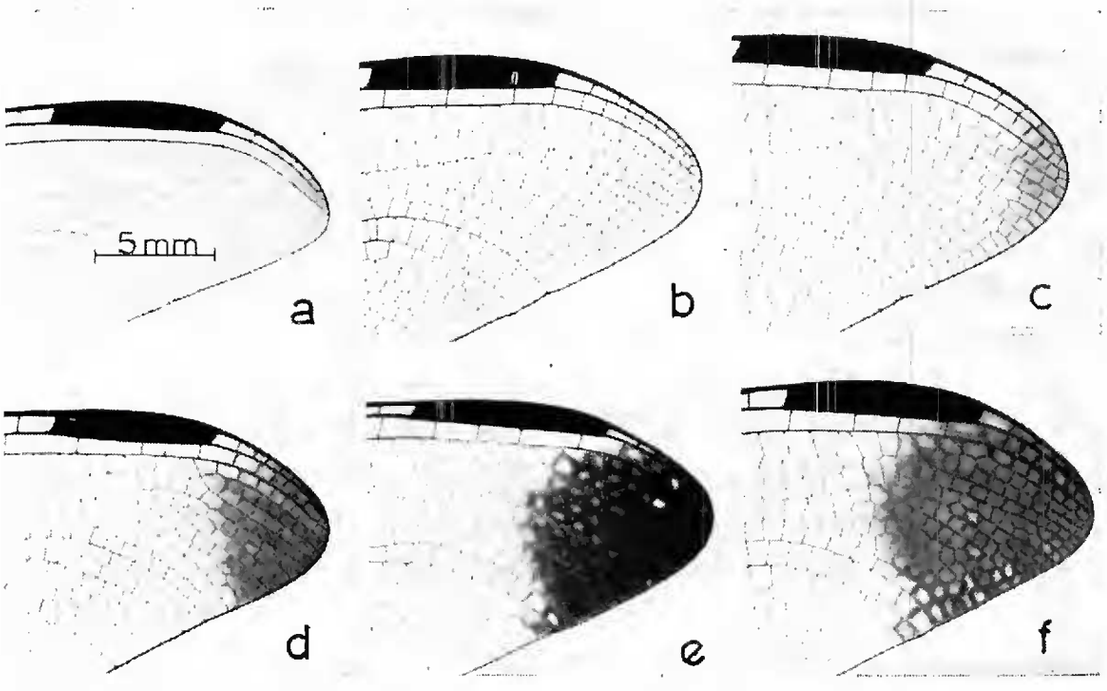

FIG. 2. Coded examples for wingtip coloration width and length. a = present, b = 1 (absent), c = 3, d = 5, e = 8, f = 9 (brown to midpoint of pterostigma).

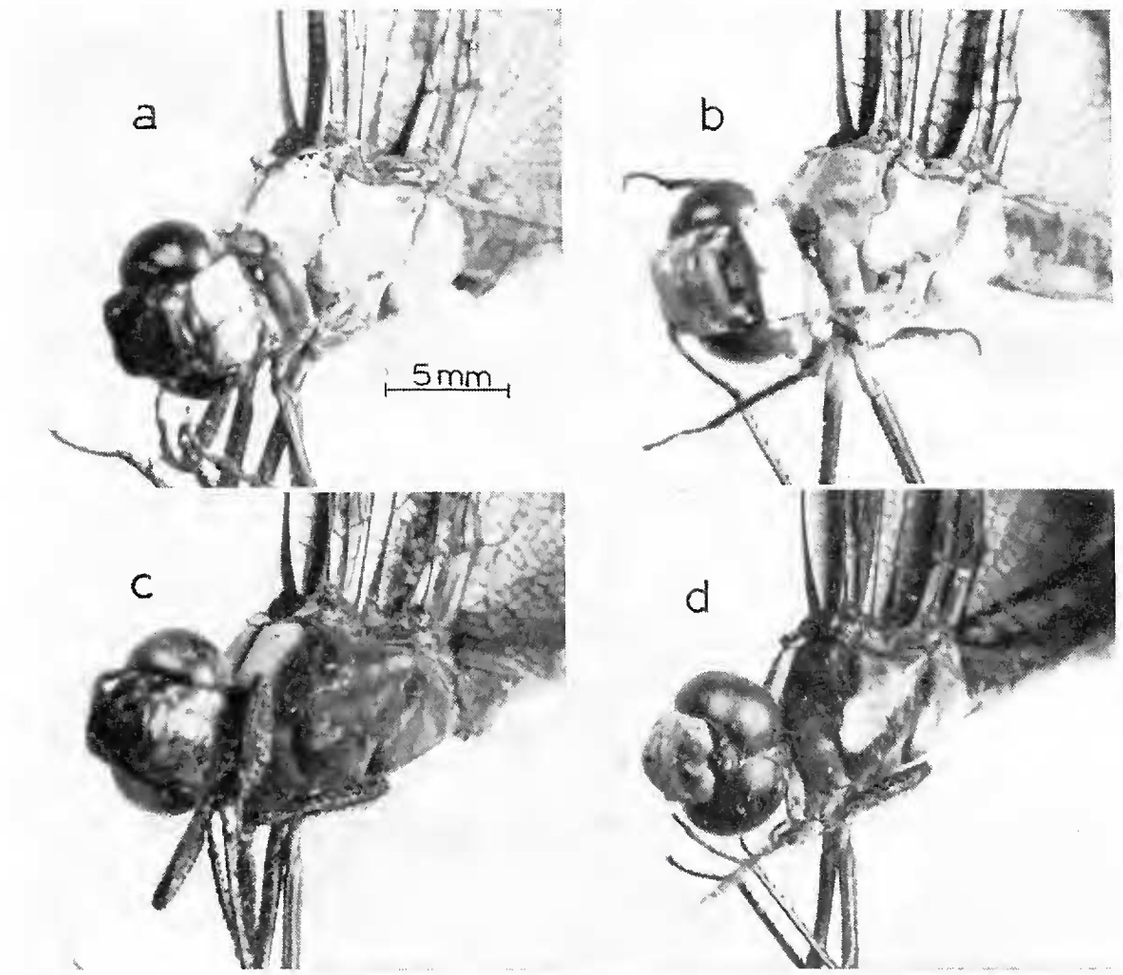

FIG. 3. Thoracic patterns of *Libellula luctuosa*. a. male Colorado River morph (Blythe, Riverside Co., Calif.), b. female Colorado River morph (Blythe, Riverside Co., Calif.), c. male (Peña Blanca Lake, Santa Cruz Co., Ariz.), d. female (Peña Blanca Lake, Santa Cruz Co., Ariz.). Both sexes of the odiosa and luctuosa morphs have thoracic patterns as in c. and d.

phenotype intergrades with nominate *L. luctuosa* west of the Mississippi River and in the Edwards Plateau region of central Texas. Allopatric populations of the odiosa phenotype also occur within the Central Valley and foothills of the Sierra Nevada of California north of the Tehachapi Mountains to southern Oregon. A single male from Robson, British Columbia, has no clearing in the hind wing band and is classified in this study as nominate *L. luctuosa*.[1]

A third group of populations inhabits the Sonoran, Colorado, and Mojave deserts and is separated from the Central Valley populations by the Tehachapi Mountains, and from the southeastern Arizona populations by the eastern edge of the Sonoran Desert. This phenotype, known

[1] Both the British Columbia (American Museum of Natural History) and Oregon males (H. G. Dyar collection at the U.S. National Museum) are probably mislabeled. The Oregon locale is Crater Lake, 29 July 1920. *Libellula luctuosa* has been taken at the Klamath River immediately south of the Oregon border.

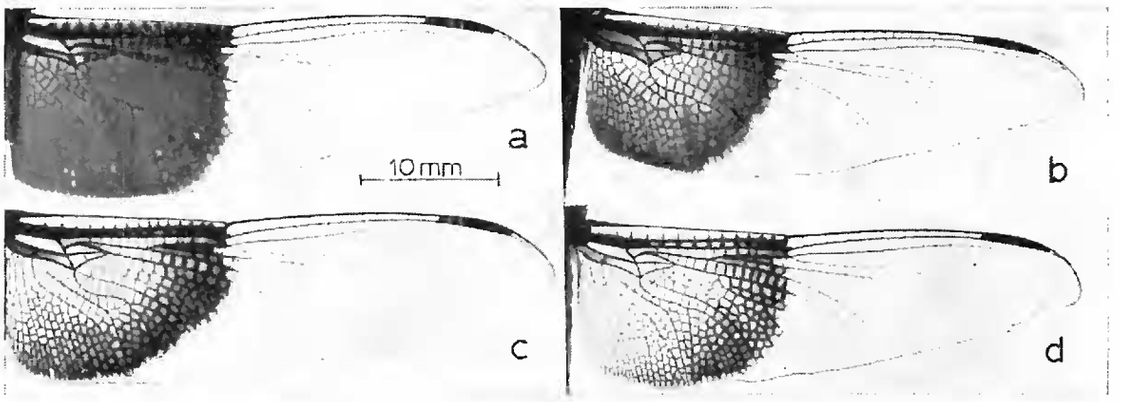

FIG. 4. Coded examples for hindwing fenestration. a = 1 (no clearing), b = 3, c = 8, d = 12 (maximum extent of hindwing clearing).

hereafter as the Colorado River (or desert) morph, has the thorax completely pruinose blue in males (Fig. 3a), while pruinosity is localized on the mesepisternum in luctuosa and odiosa phenotypes (Fig. 3c). Mature females possess a violaceous pruinosity over the typical brown areas found in odiosa and luctuosa (Fig. 3b and 3d). The hind wing bands are more fenestrated than those of most odiosa, and the females always possess dark brown wing tips (Fig. 2e and 2f), sometimes present in odiosa and luctuosa females. The pale color of individuals from desert areas agrees with Gloger's rule (Mayr, 1963).

The author subjected the data from nearly 200 *Libellula luctuosa*, intuitively classified by the characters discussed above and enumerated in Table 1, to the following multivariate means of classification: 1) principal component analysis, 2) step-wise discriminant analysis, 3) linear discriminant analysis, and 4) hierarchical numerical taxonomic methods. *Libellula luctuosa*, with its broad spectrum of geographic variation and widespread distribution (Fig. 1), is a suitable species for this study. The purpose of the study was to test compatibility of results of various methods and to compare those results with the author's intuitive concept of recognizing the species as three geographical entities, as well as to describe and explain patterns of morphological variation.

METHODS AND MATERIALS

Variation in *L. luctuosa* was analyzed using 116 males and 72 females from 26 states and provinces shown in Figure 1. A total of 10 specimens (five males and five females), if available, was chosen from each state. More individuals were chosen from Texas due to its size, and from California and Arizona because the Colorado River morph is apparently restricted to those two states.

TABLE 1. Characters.

A. Measured Characters

1. **HINDWING:** Linear distance from humeral plate to tip of wing.
2. **PTEROSTIGMA:** Linear distance along costal margin of hindwing.
3. **INTERPOLATED ANKLE CELLS IN ANAL LOOP:** Number of enclosed cells between veins A_1 and A_2 but not adjacent to those veins. Ankle cells lie on either side of the midrib.
4. **GAFF:** Linear distance between hind angle of hindwing triangle and heel of anal loop.
5. **SOLE:** Linear distance from heel to toe of anal loop.
6. **MIDDORSAL THORACIC STRIPE WIDTH:** Width measured near the antealar sinuses. Absence of stripe, as in Colorado River phenotype males, $= 1$; for other individuals, a value of 1 was added to the width measurement.
7. **METATHORACIC FEMUR:** Length along ventral side.
8. **METATHORACIC TIBIA:** Length along ventral side from distal end to concavity before articulation point.
9. **ABDOMINAL SEGMENT 5:** Length along lateral carina.
10. **SUPERIOR CAUDAL APPENDAGES:** Length along dorsal surface of superior caudal appendages of males or cerci of females.

B. Coded Characters

11. **WINGTIP COLORATION WIDTH:** Brown in anterior one-fourth of wingtip. Coded 1 (absent) (FIG. 2b) or 2 (present) (FIG. 2a). Present only on some male Colorado River morphs.
12. **WINGTIP COLORATION LENGTH:** Length of brown on wingtip. Character states ranged from 1 (no brown) to 9 (brown to midpoint of pterostigma) (FIG. 2b–f).
13. **THORACIC COLORATION:** Absence (1) or presence (2) of pale mazarine (pruinose) blue on sides of synthorax (mesepimeron, metepisternum, metepimeron). Present only on male Colorado River phenotype (FIG. 3a).
14. **FOREWING FENESTRATION:** Absence (1) or presence (2) of clearing within brown forewing band of males. As almost all individuals possessed some degree of clearing, this character was included to test its importance relative to other characters. The light area in the mid-basal space in fore- and hindwings is typical of all populations and was not included.
15. **HIND WING FENESTRATIONS:** Degree of fenestration in the hindwing band, coded 1 (no fenestration posterior to mid-basal space) to 12 (hindwing clear from midbasal space to two or three cells anterior to toe of anal loop).
16. **FRONS:** Color coded: 1) maize yellow, 2) aniline yellow, 3) Isabella color, dark olive buff, or deep chrome, 4) olive brown or dark olive, and 5) metallic black. Frons coded by the color covering 50% or more of its surface.
17. **ANTECLYPEUS:** Color coded as above.

(TABLE 1. Cont.)

18. **LABIUM:** Color coded as above.
19. **MIDDORSAL THORACIC STRIPE COLOR:** Coded as for frons with one additional color state: 6) pale mazarine (pruinose) blue.
20. **PROTHORACIC FEMUR:** Color of ventral sides coded as for middorsal thoracic stripe color.

Appropriate portions of the U.C.L.A. BIOMED program series as modified for the U.C.B. computer center were used in this study. Patterns of variation were also analyzed by numerical taxonomic methods (Sneath and Sokal, 1973), using the NT-PAK (Numerical Taxonomy Package by W. W. Moss and L. N. Bell). The data were run on the CDC-6400 computer at the University of California Computer Center at Berkeley. Because fully mature *L. luctuosa* are sexually dimorphic, males and females were run separately.

CHARACTERS

Only mature or juvenile natural adult specimens were used in this study. Presence of pruinosity, a characteristic whitish bloom present on many male Libellulidae, was the basis for selecting mature males. Pruinosity is often destroyed on preserved specimens by heat (Pinhey, 1951), chemical solvents, or leakage of body oils. Excessive heat can also cause the wings and body to glisten like tenerals immediately after eclosion. The types of *L. odiosa* are in this condition and were so described by Hagen (1861): "Entirely brassy-black . . . ," and Muttkowski (1908) separated *L. odiosa* from *L. luctuosa* by this means. No females with glistening wing membranes nor teneral specimens were used because their coloration and maculation differ from mature adults and because many tenerals were in poor morphological condition, preventing accurate measurements. Juvenile specimens possess a hard cuticle but lack fully developed pruinosity patterns. The accessory genitalia of males, usually of great taxonomic importance in dragonflies, showed no consistent differences, and were not used. Post-mortem color changes in a few poorly preserved specimens, especially females, were recorded, even though these changes were probably unnatural. Missing characters for about 6% of the specimens were substituted by values from similar specimens of the same size. These conditions constitute some of the inherent errors common to any phenetic study of organisms.

All continuous characters except hind wing length were measured to the nearest 0.01 mm using an ocular micrometer. Repeated measure-

TABLE 2. Factor Loadings for Characters of *L. luctuosa*. Important loadings ($-.200 > \alpha > .200$) are underlined.

	Males Principal Component			Females Principal Component	
Character	I	II	III	I	II
Hindwing	<u>−.239</u>	<u>−.385</u>	.099	<u>−.376</u>	.180
Pterostigma	<u>−.325</u>	−.117	−.004	<u>−.376</u>	.136
Ankle Cells	.047	<u>−.317</u>	<u>−.277</u>	−.158	.150
Gaff	−.134	<u>−.263</u>	.025	<u>−.269</u>	.101
Sole	.136	<u>−.431</u>	.089	<u>−.131</u>	<u>.349</u>
Middorsal Stripe Width	<u>.332</u>	<u>−.225</u>	−.033	−.188	<u>−.123</u>
Femur	<u>−.258</u>	<u>−.275</u>	.093	<u>−.360</u>	.129
Tibia	<u>−.302</u>	−.181	.031	<u>−.318</u>	.097
Abdominal Segment 5 Length	<u>−.226</u>	<u>−.264</u>	.033	<u>−.312</u>	<u>.203</u>
Caudal Appendages	<u>−.182</u>	<u>−.267</u>	−.086	−.113	<u>.145</u>
Wingtip Width	−.190	<u>.185</u>	.001	—	—
Wingtip Length	.098	−.063	.013	−.156	<u>−.358</u>
Thoracic Color	<u>−.362</u>	<u>.234</u>	−.103	—	—
FW Fenestration	<u>−.041</u>	<u>.020</u>	−.021	−.034	−.056
HW Fenestration	<u>−.335</u>	.117	−.030	<u>−.279</u>	−.172
Frons	<u>−.076</u>	.052	<u>.620</u>	<u>.188</u>	<u>.379</u>
Clypeus	.171	−.119	<u>.296</u>	<u>.215</u>	<u>.346</u>
Labium	<u>.221</u>	−.181	<u>.290</u>	<u>.017</u>	<u>.229</u>
Middorsal Stripe Color	<u>−.263</u>	<u>.101</u>	<u>.297</u>	<u>.141</u>	<u>.346</u>
Prothoracic Leg Color	.048	.123	<u>.478</u>	<u>.149</u>	<u>.306</u>
Percentage of Total Variation	28%	14%	8%	27%	18%

ments revealed an error of about 5%. A standard millimeter rule was used for the hind wing measurement. Of the 20 characters listed in Table 2, only 18 were recorded for females. The blue thoracic coloration and wingtip width characters were not present in females. Color terminology is after Ridgway (1912) and morphological terminology is after Needham and Westfall (1955).

ANALYTICAL TECHNIQUES

Multivariate techniques simultaneously compare all character values over each OTU (Operational Taxonomic Unit). Many authors have utilized these techniques for biological material, including Moss (1967), Moulton (1973) and Rohlf (1968) for principal components, Barlow, Graham and Adisoemarto (1969) and Bigelow and Reimer (1954) for linear discriminant analysis, and Rohwer and Kilgore (1973) and Rohwer (1972) using both methods. Numerical taxonomic methods are elaborated in Sneath and Sokal (1973).

Principal component analysis was first used because a prior assignment of specimens into arbitrary reference groups is not necessary. Combinations of characters representing OTU's are transformed to uncorrelated axes represented in an n-dimensional hyperspace. Principal axes are then established within this hyperspace so that each OTU can be expressed by its spatial relationship to n-number of components. The first component accounts for the greatest percentage of variation, the second accounts for the next greatest percentage of variation, and so on until all variation over all OTU's is expressed. Factor loadings for all characters provide a basis for determining which characters are discriminatory and which are uncorrelated. One hundred sixteen males (20 characters) and 72 females (18 characters) were analyzed.

Step-wise discriminant and linear discriminant analysis require an assignment of OTU's into arbitrary groups of known entities. The first involves a multiple discriminant analysis in a step-wise manner, whereby variables having the highest F-values (discriminatory ratio value of each value compared to the sum values of previously entered variables) are repeatedly selected for discrimination between groups. Each succeeding step utilizes the preceding combination of variables, adding another variable either with the next higher F-value, or with an F-value which, when combined with the previous set of variables, yields the greatest separation of groups. With each iteration, variables yielding the highest F-values are determined and the overlap and misclassification of OTU's between groups are recorded. Since many museum specimens are affected by post-mortem color and pruinosity pattern changes, only continuous characters were utilized to determine their value in classifying OTU's. The data were run in two groups for each sex, one with 10 characters (hindwing, pterostigma, ankle cells, gaff, sole, middorsal thoracic stripe width, femur, tibia, abdominal segment 5, and superior caudal appendages), the other including an eleventh character, hindwing fenestration, to determine its importance in further separating the groups. Twenty-five specimens from each phenotypic

group, if available, were compared. Since only 13 female Colorado River females were available, 32 odiosa females were included so that the total number of OTU's would approximate that for the males.

Linear discriminant analysis for two groups defines an axis through multidimensional clusters of OTU's resulting in a maximum separation of the clusters. Coefficients for each character are provided and these multiplied by their respective character value and summed, resulting in a single score or z-value for each OTU which can then be plotted on a histogram. From the histogram, unassigned specimens can be plotted in the same manner prescribed for reference specimens, and their position relative to the two end groups determined. Linear discriminant analysis provides an objective means by which unknown OTU's can be classified; only specimens with z-values intermediate between the two reference samples cannot with assurance be assigned to either group.

Calculation of z-values for each taxon is most advantageous when only a few characters are used. The use of many characters complicates the computational procedure and defeats the simplicity of the method. Bicoded qualitative characters which are easily discernible need not be used, since the observer can easily segregate his samples into like phena. Therefore, only four continuous variables were used: sole length, hindwing fenestration, middorsal thoracic stripe width, and femur length. The four characters repeatedly ranked with high F-values above the other seven variables in the step-wise discriminant program.

The NT 11 package used in this study performs character standardization by variance, ranking of similarity coefficients, cluster analysis using unweighted pair group averages (UPGA), histogram, and minimally connected network (Primnet: Prim, 1957). The phenograms were based on average linkage and represent a one-dimensional clustering of OTU's by similarity coefficients. The Primnets represent a one-dimensional linkage of OTU's by lines of similarity based on taxonomic distance. The longer the line between OTU's, the less similar the OTU's. The relative position of non-linked OTU's to each other is arbitrary and does not denote similarity.

RESULTS

Principal Component Analysis.—The spatial relationships of the three phenotypes are depicted in Figures 5 and 6 for males and Figure 7 for females. The squares, triangles, and circles throughout this study represent luctuosa, odiosa, and Colorado River morphs, respectively. The first scattergram for each sex shows the OTU's in relation to the first and second components; the second set for males shows the OTU's in

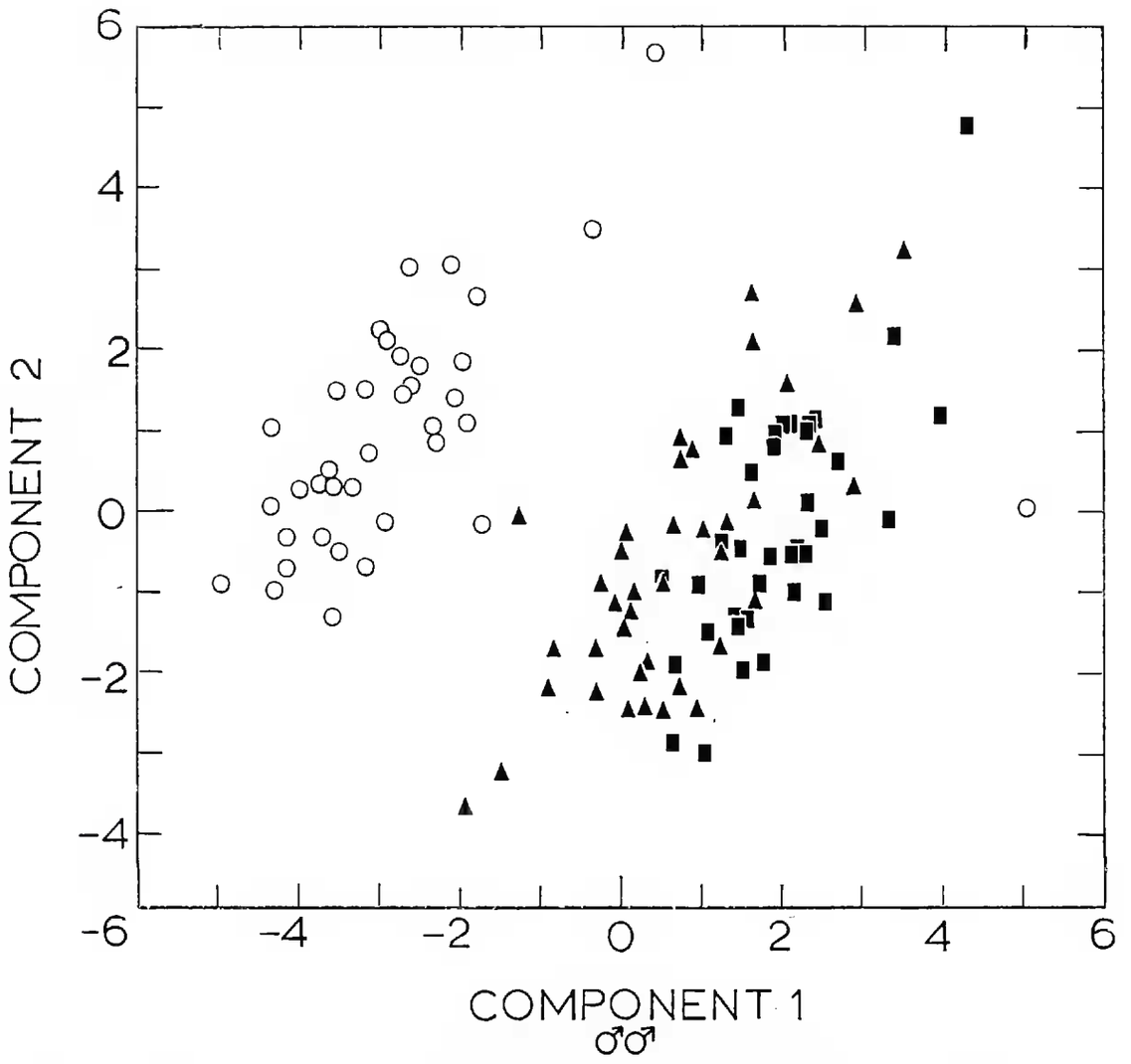

FIG. 5 (above) and FIG. 6 (right). Scattergram of principal component scores for males. Open circles = Colorado River morphs, solid triangles = odiosa, and solid squares = eastern luctuosa. Distribution of symbols indicates overall phenetic similarity to other morphs. Continuous measured characters (Table 2) are primarily responsible for separation of Colorado River and odiosa-luctuosa morphs in Fig. 5. Color-coded characters (Table 2) are primarily responsible for separation of morphs in Fig. 6.

relation to the first and third components. Clusters of OTU's for males are defined by combining both graphs.

Factor loadings for males (20 characters) are listed in Table 2. Only wingtip color length and forewing fenestration resulted in uniformly low values ($-.100 < \alpha < .100$) for the first three components, indicating that these characters were not important in segregating the groups. A combination of continuous and coded characters was important in separating the various groups. Measured characters primarily showed the highest factor loadings ($-.300 > \alpha > .300$) on the first two components. Highest coefficients were for hindwing (component 2), ptero-

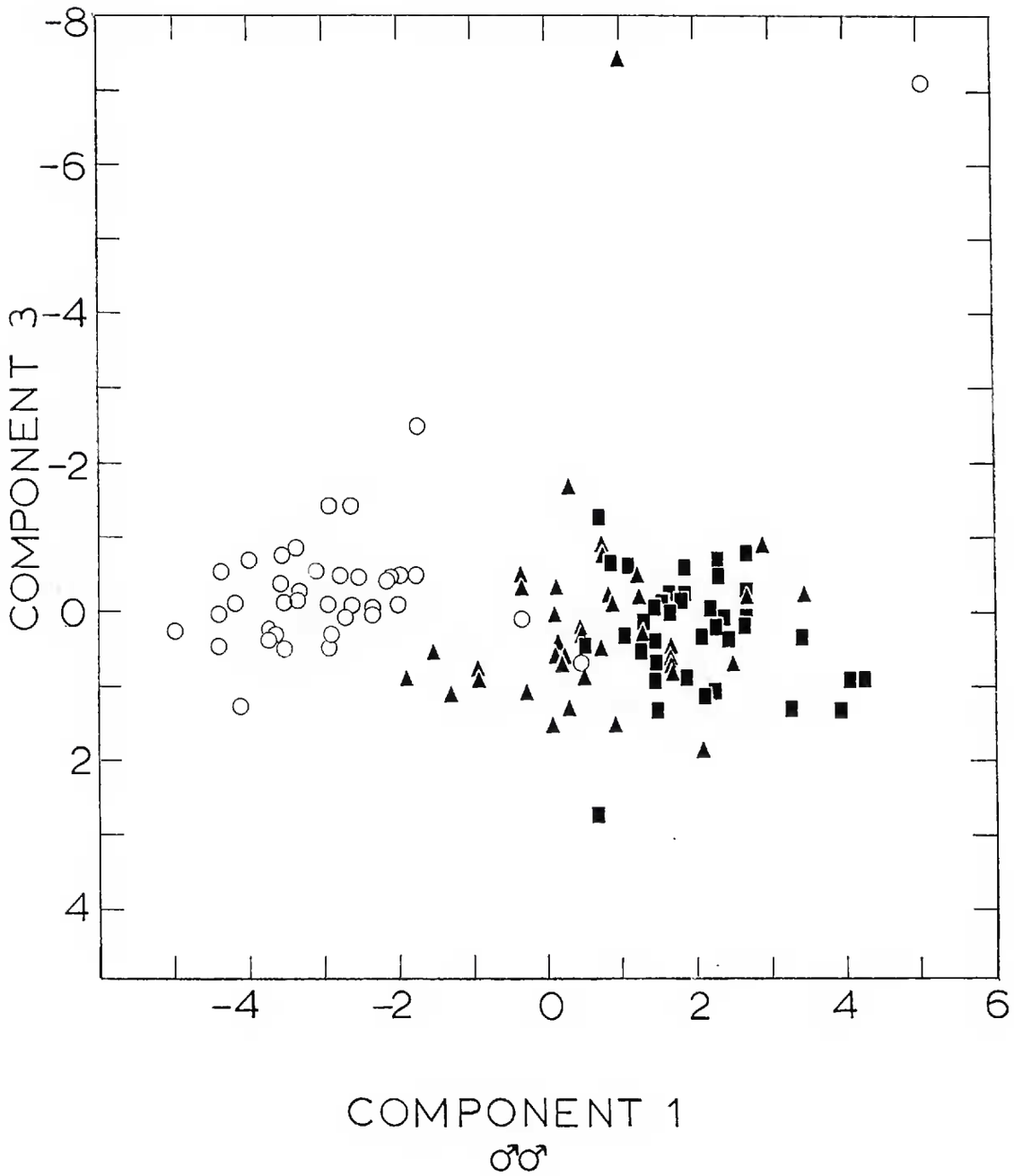

FIG. 6. See Fig. 5 for explanation.

stigma (component 1), sole (component 2), middorsal stripe width (component 1), tibia (component 1), thoracic color (component 1), and hindwing fenestration (component 1). Character coefficients on the third component revealed that color variation (frons, clypeus, labium, middorsal stripe, and prothoracic leg color) was mostly responsible for separating the groups; the ankle cell number was the only measured character with high value. The first three components accounted for 28%, 14%, and 8%, respectively, or a total of 50% of the variation. Components 4 through 18 accounted for the remaining 50%, but each component averaged 3.3% (range = 6% to 1%).

The scattergrams for males show luctuosa and odiosa to form a single cluster, although individuals of odiosa approach Colorado River individuals in appearance. The third component (Fig. 6) was minimally useful in further delineating these groups. Three males were misclassified: one odiosa clustered with the Colorado River forms (components 1 and 2); another odiosa clustered correctly in relation to components 1 and 2, but was greatly separated by component 3; a single Colorado River individual clustered with the latter odiosa specimen, forming a separate subgroup. The last two males were juvenile, which probably accounts for their aberrant location in the scattergram. Since the third component represents primarily color variation, color and maculation of these juveniles are probably responsible for their misclassification. Blue pruinosity on the juvenile Colorado River morph had not obscured the yellow middorsal stripe and brown and yellow sides of the synthorax. In addition, both juveniles had an olive-brown frons and lighter facial colors, instead of a black frons and darker facial colors common to all other individuals. The important factor loadings (Table 2) for these characters on component 3 probably contributed to their misclassification. The thoracic color coefficient was also relatively high on components 1 and 2, and it probably contributed to the odd placement of the juvenile Colorado River male, since it was the only Colorado River morph lacking the blue pruinosity on the sides of the pterothorax. Of the 116 males, 2% were misclassified according to the author's subjective treatment of the groups. The odiosa and luctuosa phenotypes are not phenotypically distinct enough to warrant their status as separate taxa.

Results for the females were similar to those for the males, except that the 13 Colorado River females did not separate as clearly. Factor loadings for the first two components (Table 2) show forewing fenestration, as in the males, to be least important in separating the phena. All other characters were important in explaining the variation; however, higher coefficients ($-.300 > \propto > .300$) prevailed for continuous characters (hindwing, pterostigma, femur, tibia, and abdomen 5) on component 1, while the same range of values predominated for coded character coefficients (wingtip length, frons, clypeus, middorsal stripe color, and prothoracic leg color) for component 2. Only one measured character (sole) resulted in a high value on component 2. Because body color patterns in female *L. luctuosa* vary more than in mature males, it is logical to expect greater importance for coded color characters. The southwestern desert forms are lighter than their eastern counterparts. The other components were not useful in further delineating between

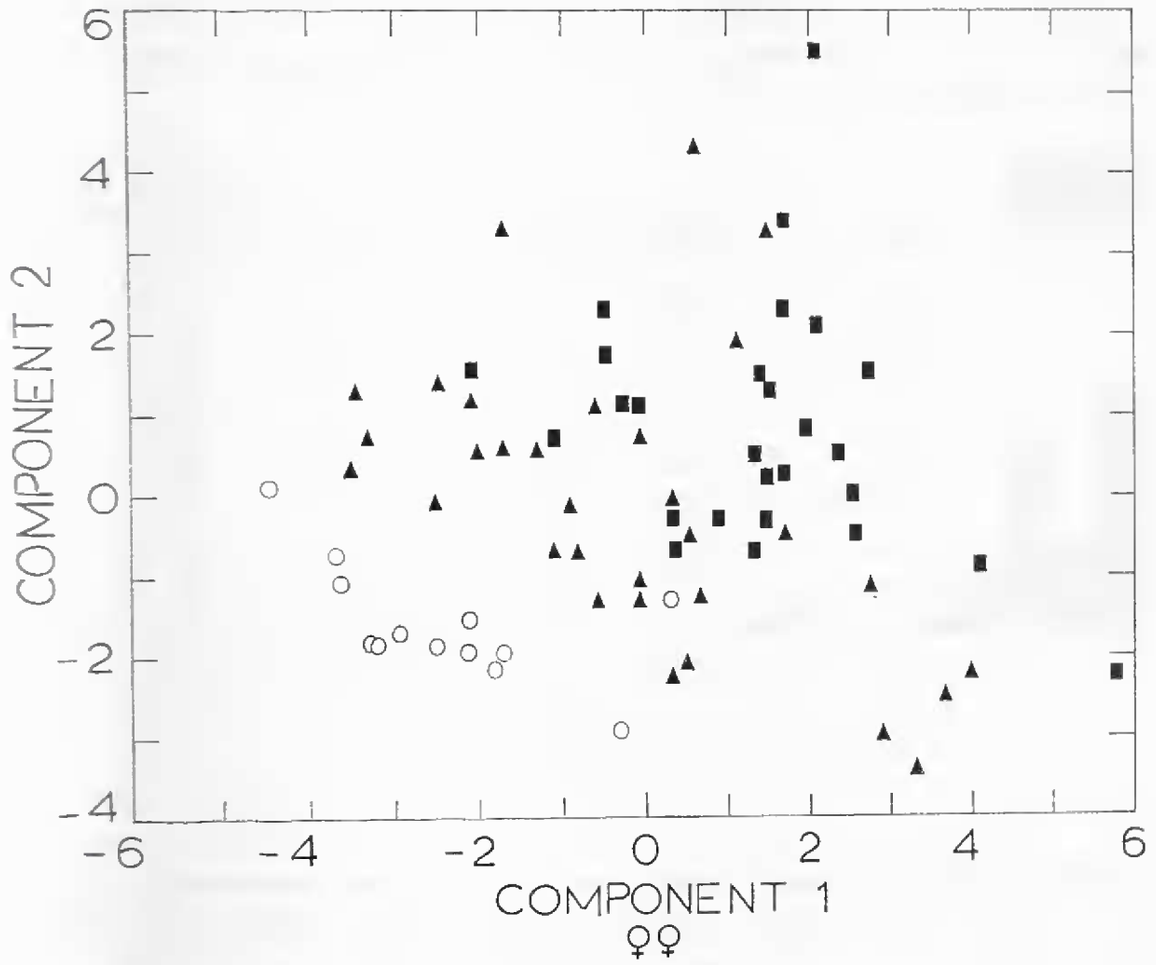

FIG. 7. Scattergram of principal component scores for females. See FIG. 5 and 6 for explanation of symbols.

groups. The first and second components accounted for a total of 45% of the variation. Components 3 through 17 accounted for the remaining 55%, with each component averaging 3.4% (range = 10% to 1%) of the variation.

As in males, there is no clear separation between odiosa and luctuosa morphs. Of the 13 desert OTU's, one clustered with odiosa (Fig. 7). This specimen was the only juvenile female of the group (lacking most of the violaceous pruinosity on thorax and abdomen), but otherwise its color and maculation did not differ appreciably from the other 12. However, it and one other female were the smallest specimens of the group. The other female was mature, but its location in Figure 7 ($PC_1 = -.367$, $PC_2 = -.288$) places it near the juvenile. Phenetic gaps between the desert and odiosa-luctuosa phenotypes are not as great as for the males.

Step-wise Discriminant Analysis.—Of the 10 or 11 measured characters used, only four, with two exceptions, repeatedly emerged with high

TABLE 3. Classification of Assigned OTU's into Respective Groups with 10 and 11 Characters by Step-wise Discriminant Analysis—Males (M) and Females (F).

Number of Characters		Morphs	luctuosa	odiosa	Colo. Riv.	Total	Number Misclassified	Percent Misclassified
M	10	luctuosa	20	5	0	25	5	20
		odiosa	7	16	2	25	9	36
		Colo. Riv.	1	0	24	25	1	4
M	11	luctuosa	24	1	0	25	1	4
		odiosa	5	19	1	25	6	24
		Colo. Riv.	0	1	24	25	1	4
F	10	luctuosa	20	4	1	25	5	20
		odiosa	8	22	2	32	10	31
		Colo. Riv.	1	1	11	13	2	15
F	11	luctuosa	23	2	0	25	2	8
		odiosa	6	24	2	32	8	25
		Colo. Riv.	0	1	12	13	1	7

F-values. Highest of the F-values was for hindwing fenestration ($14.36 < F < 706.18$), followed by middorsal stripe width ($9.16 < F < 58.82$), then tibia ($8.33 < F < 37.46$), and finally sole length ($0.90 < F < 28.17$). Abdominal segment length ($F = 4.02$) and femur length ($F = 7.21$) were the only other characters selected in the first three steps.

Classification of assigned specimens into their respective groups showed odiosa morphs to be more frequently misclassified (up to 36%) than the other two groups. This shows the odiosa sample to be a more heterogeneous group, a conclusion supported by the high variability of hindwing fenestration. Table 3 shows that most of the misclassified odiosa were placed with luctuosa.

Linear Discriminant Analysis.—Principal component analysis revealed insignificant differences between odiosa and luctuosa, so these forms were treated as one group in this analysis. The four characters selected by step-wise discriminant analysis were used to classify unassigned *L. luctuosa*. A histogram of z-values for males, using the following four discriminant function coefficients, is shown in Fig. 8: sole = .036, hindwing fenestration = –.006, middorsal thoracic stripe width = .116, and tibia length = –.025. The mean z-value for the odiosa-luctuosa group was .208, and for the Colorado River males, .105. While the mean of the two was .156, it could not be used to separate the groups, because four odiosa had values less than .156; but a critical z-value of

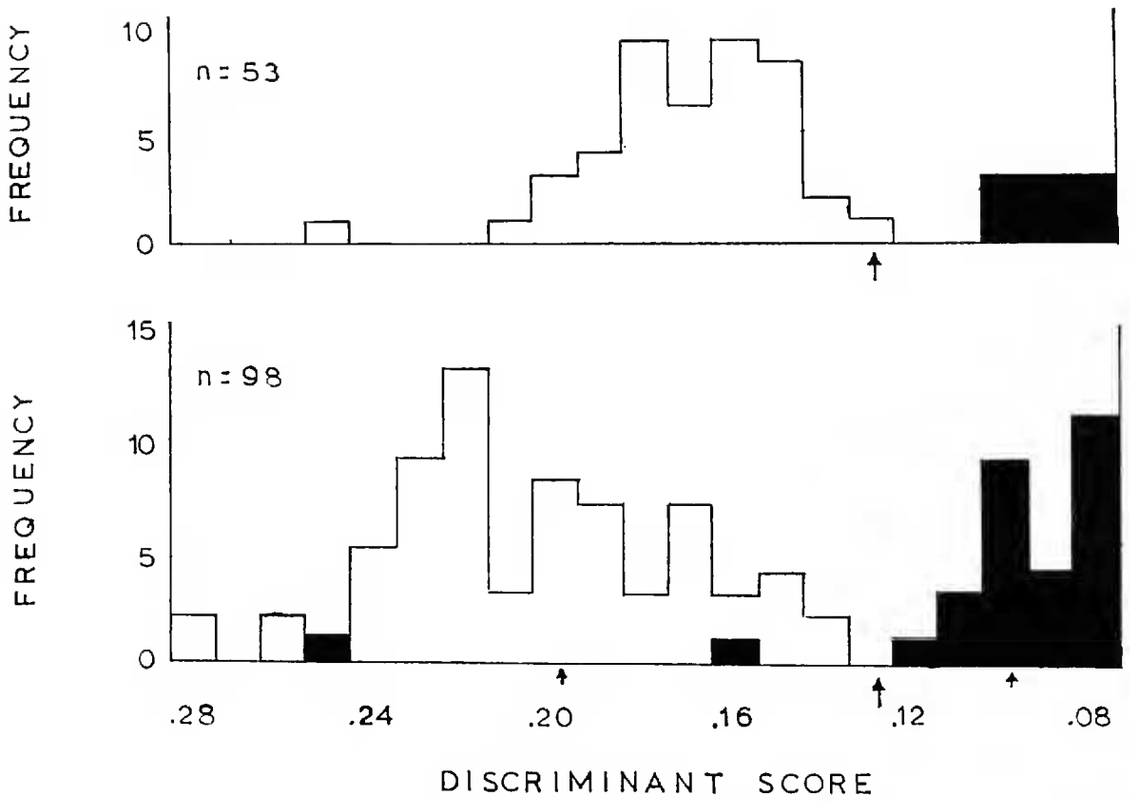

FIG. 8. Histograms of discriminant scores for reference males (below) and unassigned males (above). White = luctuosa (reference) and odiosa (unassigned), black = Colorado River morph. Means for all groups are marked by small arrows; larger medial arrows indicate critical z-value.

.130 segregated the two reference samples. Therefore, OTU's with a $z > .130$ are classified as odiosa-luctuosa phenotypes, while OTU's with $z < .130$ are classified as Colorado River morphs. Two misclassified Colorado River morphs had scores of .255 and .164 (Fig. 8). The former specimen was the juvenile previously misclassified by principal component analysis. The second male was similar to the others: no reason for its misclassification is apparent. As with principal component analysis, 2% of the reference males were misclassified.

Fifty-three unassigned male *L. luctuosa* comprising mostly of odiosa phenotypes from Texas, New Mexico, Arizona, California (Central Valley), as well as nine Colorado River phenotypes from Yuma and Phoenix, were plotted using discriminant constants for reference males (Fig. 8). All but one of the specimens were classified correctly, though the odiosa phenotypes are heavily skewed to the right of the reference males. The odiosa sample lacked any eastern luctuosa phenotypes and indicates that the odiosa morphs are intermediate between the end groups but are more similar to nominate *L. luctuosa* than to the Colorado River phenotype. Only one odiosa morph had a z-value falling within the critical range and could not be classified with certainty.

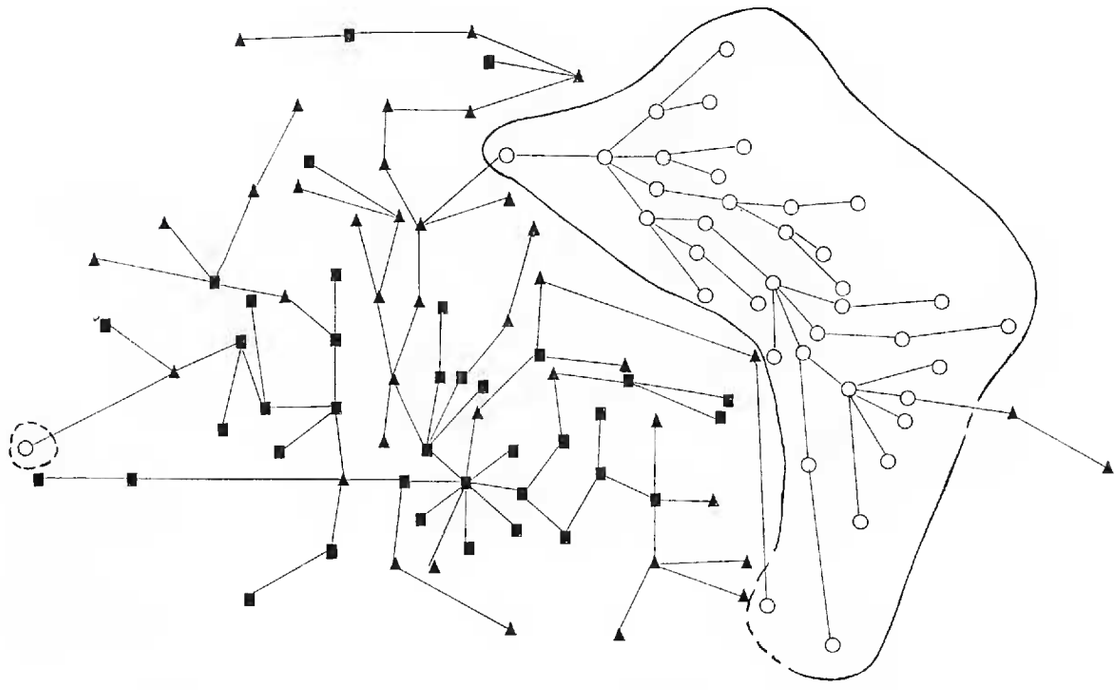

FIG. 9. Minimally connected network (Primnet) for males (n = 116) based on taxonomic distance. Open circles = Colorado River morphs, triangles = odiosa, squares = luctuosa. Connecting lines between OTU's indicate phenetic similarity, placement of non-connected OTU's is arbitrary and indicates no phenetic relationship. Superimposed solid curvilinear lines indicate *a priori* clusterings of Colorado River morphs, dotted lines indicate those misclassified. See text for further explanation.

Linear discriminant values for females were not useful in separating Colorado River forms from odiosa-luctuosa. Only 13 Colorado River females were available for the study, and four of them were classified as intermediate between odiosa-luctuosa phenotypes. Two others fall on the critical z-value (between –.39 and –.41), and the remaining seven form one end group. Of the 70 females, 8% were misclassified, but 46% of the Colorado River females were misclassified, compared to 5% for males.

Numerical Taxonomic Results.—Primnets for males and females are shown in Figures 9 and 10. The curvilinear lines superimposed over the Colorado River morphs (open circles) indicate the author's subjective classification of this group; dotted lines indicate misclassifications in the Primnet. The results are largely concordant with principal component and linear discriminant analysis. Little distinction was made between odiosa and luctuosa males (Fig. 9); there were several connections between the two. The Colorado River morphs are on a separate sidebranch, with two odiosa OTU's connected to it. On the other hand, two Colorado River morphs are connected to odiosa OTU's in remote

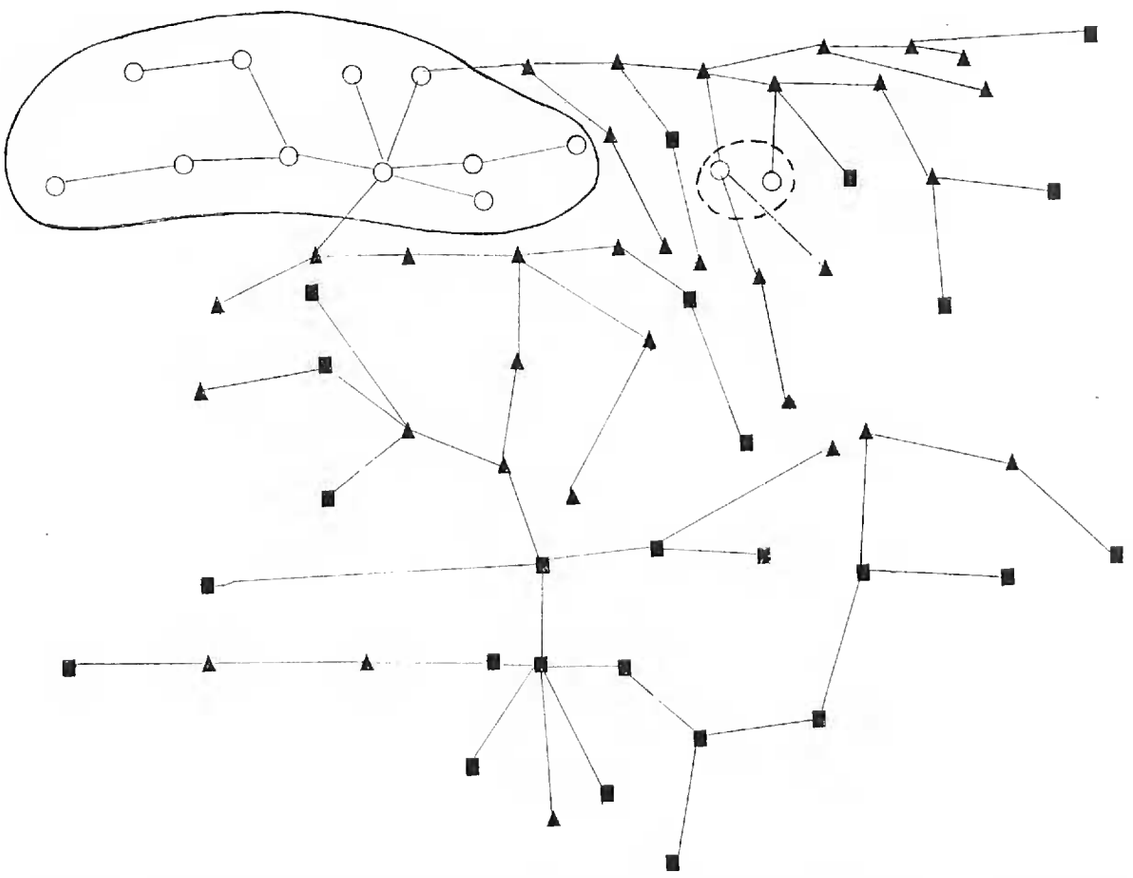

FIG. 10. Minimally connected network (Primnet) for females (n = 70) based on taxonomic distance. Symbols and designations as for Figure 9. See text for further explanation.

parts of the network. One of these is the same juvenile specimen misclassified by principal component and linear discriminant analysis. The other specimen was not misclassified by any of the previous methods, and the reason for its misclassification is unclear. The taxonomic distance between OTU's is relatively uniform, with a range of 0.38 to 1.96, with 104 OTU's having values less than 1.00. If odiosa and luctuosa morphs are considered the same, only two Colorado River morphs out of 116 OTU's (2%) were misclassified according to *a priori* treatment by the author.

Classification of the females (Fig. 10) was similar to preceding results but was much more confused. The Colorado River phenotypes do not form a separate sidebranch, but are linked to odiosa OTU's in different parts of the network. Two females of the Colorado River morph are connected only to odiosa phenotypes, the same two specimens misclassified by principal component and linear discriminant analysis. Taxonomic distances range from 0.33 to 2.10, but 65 of the OTU's had distances less than 1.00. In both Primnets, no Colorado River phenotypes are connected to any eastern luctuosa morphs.

As with the previous programs, phenetic gaps between Colorado River morphs are obvious in males, but not in females. Most misclassified OTU's were identical in all methods, which indicates discrepancies in size and coloration due to juvenile condition to be the most likely causes of misclassification.

DISCUSSION

The author subjectively recognized three distinct morphs of *Libellula luctuosa*, with phenotypic intermediates occurring in the central United States. A definite phenetic gap was observed only between the highly pruinose desert populations and the less pruinose luctuosa-odiosa phenotypes. The lack of intermediate forms may be due in part to inadequate collecting, but is mostly a result of the absence of specimens from the Tehachapi Mountains of California and from the eastern edge of the Sonoran Desert in Arizona.

Results of the various analyses show a high degree of concordance in the classification of intraspecific forms. The *a priori* distinctions between odiosa and luctuosa are not salient and the names do not warrant separate taxonomic rank. The only recognizable difference between the two is the degree of clearing in the hindwing bands, but an adequate sampling of populations of the odiosa morph shows this condition to be highly variable. Within the same deme are found individuals with little or no clearing (Fig. 4a) and with hindwing bands approaching dark rings (Fig. 4d). The pruinosity patterns of male odiosa-luctuosa morphs are identical, confined largely to the mesepisternum and abdomen (Fig. 3c). The middorsal thoracic carina and mesopleural regions are always black, and the rest of the synthorax is dark brown.

The California populations of odiosa, although geographically isolated from their midwestern counterparts by the Great Basin, are not phenotypically distinct. It seems likely that the allopatric populations of odiosa at one time shared a common gene pool instead of the two forms having arisen independently of one another. Specimens from the Pacific Northwest are rare, and the two individuals from British Columbia are typical of eastern luctuosa in appearance. While this suggests that *L. luctuosa* may range across southern Canada, no specimens are known from Alberta or Saskatchewan.

No captures of *L. luctuosa* are known from Nevada (La Rivers, 1940, 1941), Utah (Brown, 1934; Larsen, 1952; Musser, 1961), Wyoming, South Dakota, or Montana (Bick and Hornuff, 1972, 1974), where its absence may be due to adverse environmental effects of temperature, precipitation, or altitude. The Colorado River forms are allopatric with

the odiosa morph, and no intermediate forms are known; but this may be due to scanty collecting in the Tehachapi Mountains and the eastern Sonoran Desert. Before the drying of the Southwest during the later Tertiary (Axelrod, 1967; Antevs, 1955), forms similar to odiosa may have inhabited the area now occupied by the desert forms. Ancestral desert forms, then, may have at one time provided a continuous distribution of odiosa morphs from California to the central United States.

Libellula luctuosa has a Nearctic distribution and does not penetrate Neotropical regions of Mexico (Calvert, 1906) or peninsular Florida (Byers, 1930). Its absence from the Neotropics and the Great Basin is probably not due to physical barriers, since *L. luctuosa* is a vagile species which regularly frequents temporary bodies of water in the Southwest.

The frequency for extensive brown wingtips in females increases from east to west, so that all Colorado River females possess the condition shown in Figures 3e and 3f. In contrast to these, 29 of 110 (26%) odiosa females west of the Mississippi River and two of 30 (6.6%) *L. luctuosa* east of the Mississippi showed wingtip coloration comparable to the desert females. The sole and metathoracic tibia length of the Colorado River morphs and eastern *L. luctuosa* show an inverse relationship in size. The hindwing width of the Colorado River forms is less than that of the eastern forms, but the metathoracic tibiae are longer in the desert forms. A t-test performed on the means of both characters for both sexes was significant at the 0.05 level. The reason for this variation is unknown, but similar patterns of geographic variation have been reported by Alpatov (1929) for honeybees and by Rensch (1943) for carabid beetles.

Thirteen of 188 OTU's (7%) were misclassified by one or more methods of analysis. Eight of those were females. Of the 13, only three were misclassified by all methods—a juvenile male Colorado River morph and two female Colorado River morphs. The male differed significantly from the others in its group because pruinosity had not yet obscured its thoracic pattern. The females were probably misclassified due to age and size differences. One was juvenile, the only one of its group in this condition, and both were relatively small—their hindwing lengths were 37 mm, compared to a mean of 39.3 mm for the entire group. One of the other males misclassified by principal component analysis was also juvenile, but no other anomalies were observed for the remaining nine specimens, and the reasons for their misclassification remain obscure.

Pruinosity patterns are responsible for the greater distinctness of male than female desert forms. The presence of pruinosity is reflected

in three male characters: thoracic coloration, width of middorsal stripe, and prothoracic leg color. The thoracic coloration in females is brown and yellow with only a violaceous pruinose tint (not present in odiosa-luctuosa phenotypes) covering the usually dark brown patterns of the thorax and abdomen (Fig. 3b). The yellow middorsal stripe is always present in all females, and the prothoracic leg color is yellow. The extensive pruinose condition of the males and, to a lesser extent, females, is probably of some adaptive significance. Unfortunately, the chemical nature and biological significance of pruinosity are unknown. Johnson (1973) speculates that "it is a process of nitrogen elimination acted on by sexual selection producing mate recognition clues." There is strong evidence that pruinosity does provide mate recognition cues (Jacobs, 1955; Johnson, 1962a, 1962b), but it is also present on old females of some species (Longfield, 1960; personal observations), so its pronounced condition on the southwestern desert forms may also indicate a physiological function. The southwestern morphs conform, as do many other invertebrates, to Gloger's rule, which states that races from cool areas are more heavily pigmented than those from warm areas. There is no undisputed explanation for this phenomenon, but solar reflectance by pale coloration may be one possibility (Bodenheimer, 1954).

ACKNOWLEDGMENTS

I wish to express my sincere thanks to John T. Doyen for his patience and guidance in answering numerous questions and for his help in completing this work. His aid with the various computer techniques, and his reading and constructive criticism of the entire manuscript are most gratefully acknowledged. Special thanks are also due Woodrow Middlekauff, Jerry A. Powell, and Dennis R. Paulson for reading the manuscript and offering constructive criticisms.

The following persons and institutions loaned specimens of *Libellula luctuosa* so that this study could be more complete: E. Aitcheson, Museum of Northern Arizona, Flagstaff; F. Byers, University of Kansas, Lawrence; O. S. Flint, United States National Museum, Washington, D.C.; L. Floge, American Museum of Natural History, New York; S. Frommer, University of California, Riverside; L. K. Gloyd, University of Michigan, Ann Arbor; F. Hasbrouck, Arizona State University, Tempe; C. L. Hogue and F. S. Truxal, Los Angeles County Museum; H. B. Leech and P. H. Arnaud, California Academy of Sciences, San Francisco; J. A. Powell and J. Chemsak, University of California, Berkeley; S. S. Roback and W. W. Moss, Academy of Natural Sciences of Philadelphia; F. G. Werner, University of Arizona, Tucson; M. J.

Westfall, Jr., University of Florida, Gainesville; and H. E. Evans of the Museum of Comparative Zoology, Cambridge, for the loan of the types of *L. odiosa.* I wish to express my sincere thanks and appreciation for their cooperation. D. R. Paulson of the University of Washington and K. Tennessen of the University of Florida kindly made available material from their private collections.

The photographs were executed by Herman G. Real; and I especially wish to thank my wife, Jo, who typed the manuscript and aided me with its completion.

Computer time was provided by a grant from the University of California Computer Center. This study was funded in part by a traineeship from the National Institutes of Health.

LITERATURE CITED

ALPATOV, W. W. 1929. Biometrical studies on variation and races of the honey bee, *Apis mellifera* L. Quart. Rev. Biol., 4: 1–58.

ANTEVS, E. 1955. Geologic-climatic dating in the West. Amer. Antiq., 20: 317–335.

AXELROD, D. I. 1967. Geologic history of the California insular flora, *in* Proceedings of the Symposium on the biology of the California islands. R. N. Philbrick, Santa Barbara Botanical Gardens, Santa Barbara, pp. 267–315.

BARLOW, C. A., GRAHAM, J. E. AND ADISOEMARTO, S. 1969. A numerical factor for the taxonomic separation of *Pterostichus pensylvanicus* and *P. adstrictus* (Coleoptera: Carabidae). Canad. Entomol., 101(12): 1315–1320.

BENNEFIELD, B. L. 1965. A taxonomic study of the subgenus *Ladona* (Odonata: Libellulidae). Univ. Kans. Sci. Bull., 45(4): 361–396.

BICK, G. H. AND HORNUFF, L. E. 1972. Odonata collected in Wyoming, South Dakota, and Nebraska. Proc. Entomol. Soc. Wash., 74(1): 1–8.

BICK, G. H. AND HORNUFF, L. E. 1974. New records of Odonata from Montana and Colorado. Proc. Entomol. Soc. Wash., 76(1): 90–93.

BIGELOW, R. S. AND REIMER, C. 1954. An application of the linear discriminant function to insect taxonomy. Canad. Entomol., 86: 69–73.

BODENHEIMER, F. S. 1954. Problems of physiology and ecology of desert animals, *in* Biology of deserts. J. L. Cloudsley-Thompson, Institute of Biology, London, pp. 162–167.

BROWN, C. J. D. 1934. A preliminary list of Utah Odonata. Occas. Pap. Mus. Zool. Univ. Mich., 291: 1–17.

BYERS, F. C. 1930. A contribution to the knowledge of Florida Odonata. Univ. Fla. Pub., 1(1): 1–327.

CALVERT, P. C. 1906. Biologia Centrali Americana: Neuroptera, R. H. Porter and Dulau Co., London, pp. 145–212.

HAGEN, H. A. 1861. Synopsis of the Neuroptera of North America. Smithsonian Miscellaneous Collections, 4(1): 1–344.

HAGEN, H. A. 1875. Synopsis of the Odonata of America. Proc. Boston Soc. Nat. Hist., 18(1): 20–96.

HUGGINS, J. R. 1927. Variations in size of *Calopteryx maculata* and a proposed new subspecies. Trans. Amer. Entomol. Soc., 52(4): 355–364.

JACOBS, M. E. 1955. Studies on territorialism and sexual selection in dragonflies. Ecology, 36: 566–586.

JOHNSON, C. F. 1962a. A study of territoriality and breeding behavior in *Pachydiplax longipennis* Burmeister (Odonata: Libellulidae), Southwestern Nat., 7(3–4): 191–197.

JOHNSON, C. F. 1962b. Reproductive isolation in damselflies and dragonflies (Order Odonata). Texas J. Sci., 14: 297–304.

JOHNSON, C. F. 1972. An analysis of geographical variation in the damselfly, *Argia apicalis* (Zygoptera: Coenagrionidae). Can. Entomol., 104(10): 1515–1527.

JOHNSON, C. F. 1973. Ovarian development and age recognition in the damselfly, *Argia moesta* (Zygoptera: Coenagrionidae). Odonatologica, 2(2): 69–81.

LA RIVERS, I. 1940. A preliminary synopsis of the dragonflies of Nevada. Pan-Pac. Entomol., 16(3): 111–123.

LA RIVERS, I. 1941. Additions to the list of Nevada dragonflies (Odonata). Entomol. News, 52(5): 126–130, 155–157.

LARSEN, W. P. 1952. The dragonflies (Anisoptera) of Utah. Unpublished master's thesis, Department of Zoology, Univ. Utah, pp. 1–95, 30 plates. Salt Lake City.

LONGFIELD, C. 1960. The British species, *in* Dragonflies. P. S. Corbet, C. Longfield, and N. W. Moore. Collins, London, pp. 11–24.

MAYR, E. 1963. Animal species and evolution. The Belknap Press, Harvard University Press, Cambridge, Mass., xiv + 797 pp.

MOSS, W. W. 1967. Some new analytic and graphic approaches to numerical taxonomy, with an example from the Dermanyssidae (Acari). Syst. Zool., 16(3): 177–208.

MOULTON, T. P. 1973. Principal component analysis of variation in form within *Oncaea conifera* Giesbrecht, 1891, a species of copepod (Crustacea). Syst. Zool., 22(2): 141–156.

MUSSER, R. J. 1961. Some noteworthy dragonfly records from Utah. Entomol. News, 72(2): 53–54.

MUSSER, R. J. 1962. Dragonfly nymphs of Utah (Odonata: Anisoptera). Univ. Utah Biol. Ser., 12(6): 1–61.

MUTTKOWSKI, R. A. 1908. Review of the dragonflies of Wisconsin. Bull. Wisc. Nat. Hist. Soc., 6(1–2): 57–127.

NEEDHAM, J. G. AND WESTFALL, M. J., JR. 1955. A manual of the dragonflies of North America (Anisoptera). Univ. Calif. Press, Berkeley, xii + 615 pp.

PINHEY, E. C. G. 1951. The dragonflies of Southern Africa. Transvaal Mus. Mem. 5, xv + 335 pp.

PRIM, R. C. 1957. Shortest connection networks and some generalizations. Bell System Technical Journal, 36: 1389–1401.

RENSCH, B. 1943. Studien über Korrelation und Klimatische Parallelitat der Rassenmerkmale von Carabus-Formen. Zool. Jahrb. (Syst.), 76: 103–170.

RIDGWAY, R. 1912. Color standards and color nomenclature. Washington, D.C., publ. by the author, iv + 43 pp., 53 plates.

RIS, F. 1910. Collections zoologiques du Baron Edm. de Selys-Longchamps. Fasc. XI, Libellulinen 3, pp. 245–384.

ROHWER, S. A. 1972. A multivariate assessment of interbreeding between the meadowlarks, *Sturnella*. Syst. Zool., 21(3): 313–338.

ROHWER, S. A. AND KILGORE, D. L., JR. 1973. Interbreeding in the arid-land foxes, *Vulpes velox* and *V. macrotis*. Syst. Zool., 22(2): 157–165.

ROHLF, F. J. 1968. Stereograms in numerical taxonomy. Syst. Zool., 17(3): 246–255.

SNEATH, P. H. A. AND SOKAL, R. R. 1973. Numerical taxonomy. W. H. Freeman and Co., San Francisco, xv + 359 pp.

SCIENTIFIC NOTE

A Female Specimen of Acanthocinus (Canonura) leechi.—In 1956 (Ann. Entomol. Soc. Amer., 49:228), Lawrence S. Dillon described a new species of *Canonura*, *C. leechi*, from a single male specimen from Jerome, Yavapai County, Arizona. The author recently collected a female in the Hualapai Mountains, Mojave County, Arizona, at black light, in pinyon pine juniper forest, at approximately 5,300 feet. Since the female has been unknown until this time, a brief description follows.

Form moderately robust, similar to the male. Pronotum at sides beneath lateral tubercles deeply punctate, lacking fuscous maculae. Antennae twice as long as body, segments with the following ratio: 1.0; .2; 1.4; 1.3; 1.2; 1.2; 1.1; 1.1; 1; 1; 1. Abdomen with fifth sternite prolonged, slightly shorter than sternites 2 through 4 combined, ovipositor strongly produced, extending 5 mm beyond tips of elytra. Length: 16 mm, not including ovipositor.

This insect is uniformly speckled with numerous small dark spots. These spots are much more discernable due to the absence of well defined hoary pubescence which is so pronounced in *A. spectabilis* (LeConte) and *A. princeps* (Walker). *A. leechi* also differs from *A. princeps* by the absence of fulvous markings on the elytra and by the color of the pubescent maculae of the pronotum. —A. E. LEWIS, *1360 Paseo Redondo, Burbank, CA 91501.*